RESPONSE TO OFFICE ACTION

A. <u>Status of the Claims</u>

Claims 2-4 and 67 are currently pending and under consideration in this case.

B. <u>Rejection Under 35 U.S.C. §112, First Paragraph, Enablement</u>

The Action rejects claims 2-4 and 67 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Action acknowledges that the specification is enabled for ". . . claims limited to stably transformed and fertile maize plants produced by a process comprising the bombardment of regenerable maize cells of the genotype (A188 X B73) with microprojectiles coated with DNA comprising a selectable marker gene, or ... [by] electroporation of maize cells with intact cell walls or immature embryos; each process followed by selection of transformed cells on the basis of marker gene expression, and regeneration of whole plants therefrom…" it is asserted, however, that enablement is lacking for the full scope of claimed subject matter. Action at page 2. Applicants respectfully traverse this rejection.

Applicants initially note that the claimed subject matter corresponds to a fertile, transgenic maize plant, the genome of which has been augmented as defined in claim 67, as well as cells, progeny and seeds thereof. The claimed subject matter is therefore a ***product***, and not a method or product by process. The relevant enablement inquiry is whether ***this product*** is enabled. MPEP 2164.01 ("Any analysis of whether a particular claim is supported by the disclosure in an application requires a determination of whether that disclosure, when filed, contained sufficient information regarding the ***subject matter of the claims*** as to enable one skilled in the pertinent art to make and use the claimed invention." (emphasis added)). Here, even if it is assumed for purpose of argument that the assertion in the Action that only two

transformation techniques are enabled *via* A188 X B73 genotype, which Applicants do not concede, this in no way suggests non-enablement of the claims, as described below.

As noted above, claims 2-4 and 67 relate to fertile transgenic plants, and the seeds, cells and progeny thereof, the genome of which has been augmented by the introduction of a DNA composition comprising a gene encoding a grain composition trait comprising a fatty acid desaturase gene. Whether the claimed plants and seeds are made by microprojectile bombardment or electroporation of a specific corn genotype, or by another method or with any other corn genotype, is irrelevant to these composition claims. The end result of any such methods is a transgenic plant having incorporated in its genome a DNA composition comprising a gene encoding a grain composition trait comprising a fatty acid desaturase gene, as defined in the claims. That is, the nature and makeup of the DNA composition recited in the claims is not dependent upon the method by which it is introduced.

The only alleged structural difference that could be relevant to the claimed product asserted in the Action is the genotype of the cells into which the DNA composition is initially introduced and from which a fertile transgenic plant is initially regenerated. However, this is also irrelevant to the claimed ***product***. Once a fertile transgenic corn plant is obtained, whether of the A188xB73 genotype or any other genotype, the DNA composition can be routinely introduced into any other corn variety of any other genotype using predictable and routine plant breeding steps, all of which have been known in the art for decades.

For instance, once a transgenic trait is produced this can be routinely introduced into any other corn genotype by the well known method of backcross conversion, *e.g.*, as described in the present specification, for instance, at page 193, line 28. Backcross conversion has been known for decades by corn plant breeders, and is used to routinely yield breeding lines and cultivars

essentially agronomically equivalent to a "recurrent" parent into which a given genetic locus is introduced from a "non-recurrent" donor parent that comprises the particular genetic locus. As an illustration of this, attached as **Exhibit A** is the reference of Briggs and Allard, 1953 (*Agron. J.* 45:131-138), describing thirty years of backcross breeding, and discussing backcrossing applications in maize. See p. 135, bottom of right column. The methodology is also described in, for example, Section N of the specification entitled "Fertility of Transgenic Plants" as follows:

> By providing fertile, transgenic offspring, the practice of the invention allows one to subsequently, through a series of breeding manipulations, move a selected gene from one corn line into an entirely different corn line without the need for further recombinant manipulation. Movement of genes between corn lines is a basic tenet of the corn breeding industry, involving simply backcrossing the corn line having the desired gene (trait). Introduced transgenes are valuable in that they behave genetically as any other corn gene and can be manipulated by breeding techniques in a manner identical to any other corn gene. Exemplary procedures of this nature have been successfully carried out by the inventors. In these backcrossing studies, the gene for resistance to the herbicide Basta®, *bar*, has been moved from two transformants derived from cell line SC716 and one transformant derived from cell line SC82 into 18 elite inbred lines by backcrossing. It is possible from these 18 inbreds to make a large number of hybrids of commercial importance. Eleven of the possible hybrids have been made and are being field tested for yield and other agronomic characteristics and herbicide tolerance. Additional backcrossing to a further 68 elite inbred lines is underway.

Thus the Specification, combined with the acknowledged corn transformation methods in the specification and routine plant breeding technology described in the specification and known in the art, clearly enables the production of any genotype of transgenic corn plant. Therefore, the specification enables every embodiment of the claimed transgenic maize plants, and seeds, cells and progeny thereof, comprising the DNA composition as defined in claim 67.

Withdrawal of the rejection is thus respectfully requested.

C. Conclusion

The examiner is invited to contact the undersigned with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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EXHIBIT A

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The Current Status of the Backcross Method of Plant Breeding[1]

F. N. Briggs and R. W. Allard[2]

THIRTY years have elapsed since Harlan and Pope (3) called attention to the value of backcrossing as a method of small-grain breeding and since an extensive backcross-breeding program was started by Briggs (1). Despite the advantages pointed out by Harlan and Pope and the successes of plant breeders who have used the method, it has not gained wide acceptance.

There appear to be several reasons for reluctance to adopt this method of breeding. One important reason is the conviction of many plant breeders that they do not possess a satisfactory recurrent parent. Another is the belief that it cannot be used for improving a variety with respect to a number of characters. The criticisms have also been made that the method is laborious and that it is valueless in dealing with quantitative characters. Further, some plant breeders have expressed the belief that it will not work with cross-pollinated crops.

In view of these and other objections to the method, it seems appropriate to discuss both the theoretical concepts basic to backcross breeding and the evidence for their validity gained from a number of backcross programs. In addition, some of the details of the use of the method at the California Station will be presented because of numerous requests for this information.

BASIC REQUIREMENTS OF BACKCROSS BREEDING

If a backcross program is to be successful, the following three requirements must be satisfied: (a) A satisfactory recurrent parent must exist; (b) it must be possible to retain a worthwhile intensity of the character under transfer through several backcrosses; and (c) the genotype of the recurrent parent must be reconstituted by a reasonable number of backcrosses executed with populations of manageable size.

The Recurrent Parent

A satisfactory recurrent parent is likely to exist in any of the crop species which have long been domesticated. In such crops, many highly efficient combinations of genes have been forged over long periods of time through the agencies of "natural selection," selection by farmers, and very recently by plant breeders. In common wheat, for example, a few highly successful varieties qualify as acceptable recurrent parents. Among the older of these varieties are Turkey (and selections from it), Marquis, Fultz and Fulcaster, Baart, and some others which have performed well over long periods of time. Included among the newer varieties with excellent performance records are Pawnee, Comanche, Thatcher, Thorne, and Ramona. A survey of our other crop species indicates that most of them also include varieties with excellent combinations of genes which qualify them as recurrent parents in backcross breeding.

In the pattern of the replacement of older varieties by new ones, the advantage of the new type has frequently been associated largely with one or two characteristics. For example, resistance to stem rust and early maturity were important features of Thatcher wheat leading to its replacement of Marquis. The most recent hard red spring-wheat varieties, such as Mida, have the added advantage of resistance to leaf rust. In the competition among hard red winter-wheat varieties much of the success of Pawnee, Comanche, Wichita, and Triumph is associated with early maturity. In addition, these varieties have more resistance than their predecessors to one or more of the major diseases or pests of wheat. Among the soft winter-wheat varieties, Thorne, Clarkan, Fairfield, and Yorkwin are less subject to lodging than their predecessors and are resistant to more races of loose smut. Apparently the most important limiting factor in the production of successful varieties is frequently susceptibility to diseases or other equally obvious deficiencies. The backcross method is well suited for effecting the small number of gene substitutions necessary to increase the usefulness of successful varieties, without the risk of breaking up the existing combinations of desirable genes which have made them outstanding in many respects.

Maintenance of the Character under Transfer

Although the backcross breeder need be concerned only with selection for the character being transferred, some of the intensity of the character may be lost even when its genetic control is predominantly monogenic. This problem prompted a series of projects at the California Station dealing with monogenic morphological characters in barley that have been particularly informative regarding problems of character transfers and ways of dealing with these problems. These projects deal with transfers of the hooded, short-haired rachilla, smooth-awn, awnless, and naked characters to Atlas.

In breeding a hooded Atlas barley, Meloy, which has an elevated hood, was chosen as the donor parent. When a few sessile-hooded types appeared in the generations following the first backcross, it was decided to breed this type. With careful selection in moderate-sized populations

[1] Contribution from the Department of Agronomy, University of California, Davis, Calif. Received for publication October 5, 1952.

[2] Professor and Associate Professor of Agronomy, respectively.

the character was maintained through five backcrosses. Similarly, the full intensity of the short-haired rachilla character as expressed in Lion and the barblessness governed by the *r* allele of Lion was transferred to Atlas without difficulty. Selection for the naked condition in a Kamananzi–Atlas backcross also presented no problem. However, it was discovered too late in this backcross program that the thin, papery lemmae typical of naked barleys are essential for easy threshing. It is possible that the heavy lemmae of Atlas could have been avoided in the derived forms had an attempt been made to do so. In breeding an awnless Atlas, Awnless C.I. 5631, a short-strawed type with club heads was chosen as the donor parent because of its nearly complete awnlessness. When plants with the height and lax heads of Atlas appeared in the segregating generations, they invariably had short awns. Selection practiced through 5 to 7 selfed generations after the first and third backcrosses have failed to retain the awnlessness characteristic of the donor parent in Atlas-like plants, and it now appears that very short awns will have to be accepted. Apparently height genes carried by Atlas affect awn length in such a way that complete awnlessness is incompatible with tallness.

Additional evidence concerning the success with which a worthwhile intensity of simply inherited characters is maintained in backcrossing is provided by some programs involving transfers of disease resistance to California cereal varieties. None of the bunt-resistant, backcross-derived wheat varieties are equal to Martin, the donor parent, in resistance. Upon artificial inoculation, Martin is nearly always completely free from infection, whereas the derived types may show as much as 3 or 4% infection. Big Club 43 permits as much as 4 to 6% infection by hessian fly compared to 2% for Dawson, the resistant parent. Similarly, few of the lines in the stem-rust-resistant varieties are equal to Hope in resistance. This loss of resistance has not limited the utility of the resistant varieties. The release of the bunt-resistant varieties coincides with the period when California changed from one of the areas most seriously affected by the disease to an area where bunt is no longer an economic problem because the resistant varieties do not allow production of sufficient inoculum to maintain the disease (16). Likewise, hessian fly has practically disappeared from California wheat fields because of its inability to live on Big Club 43. Although California formerly suffered heavy stem-rust epiphytotics at approximately 5-year intervals and lesser damage nearly every year, losses of economic proportions have not occurred in the rust-resistant varieties during the 14 years since their release.

No example is known to the authors where intraspecific backcross transfers of simply inherited characters have failed. Smith (11), however, reported a case in *Nicotiana* where the genotype of one species is epistatic to an allele from another species, and Harland has reported modifications of expression of certain alleles in interspecific backcrosses in cotton (see 4). As more transfers are made, cases of inability to transfer a worthwhile intensity of a character from one variety to another by backcrossing can be anticipated. However, sufficient evidence is at hand to indicate that such situations are not common.

The Number of Backcrosses

If backcross breeding is to succeed with certainty, the recurrent parent must be reduplicated in its essential features. While recovery of the genotype of the recurrent parent is primarily a function of the number of backcrosses, selection in the early backcross generations is effective in directing the population toward the characteristics of that parent. Additionally, sufficient plants of the recurrent parent must be used in making the final backcross to recapture any cyptic variability in the variety.

With successive backcrosses, the rate of elimination of genes of the nonrecurrent parent depends on the linkage intensity. The probability of eliminating all loci further than a given map distance from the locus for which selection is practiced is $1-(1-p)^{n-1}$, where p is the genetic distance and n is the number of backcrosses. Hence, the probability of eliminating unlinked genes or genes located 50 or more units from the desired gene is $1-(2)^{1-n}$. The mean genetical length of chromosome retained on either side of the locus for which selection is practiced is $\frac{1-(2)^{-n}}{n}$.

This approximates to 1/n when n is large. According to the above considerations, 6 backcrosses should eliminate at least 97% of the unlinked genes of the donor parent. This is an important feature of backcross breeding because the best source of the gene or genes desired may be used with little danger of introducing weaknesses characteristic of the donor parent.

If the effects of linkage and selection are ignored, the mean rate of recovery of the genotype of the recurrent parent will be described by the sequence 3/4, 7/8, 15/16, 31/32. . . . Riddle and Baker (9) have calculated that if the parents differ in 21 gene pairs, 82.3% of the population would be identical with the recurrent parent at the end of six backcrosses and that an additional 13.5% would differ by a single allele, giving a total of 95.8% closely resembling the recurrent parent. With three backcrosses and the same number of gene differences, 6.0% of the plants would be expected to be identical with and 18.2% to differ from the recurrent parent by a single allele, making a total of 24.0% which closely resemble that parent. Theoretical considerations thus suggest that six backcrosses should reconstitute quite precisely the genotype of the recurrent parent, but that three backcrosses would be inadequate to accomplish the purpose.

Six backcrosses, when coupled with rigid selection in the early generations, have proved satisfactory in a large number of backcross programs at the California Station. It is believed that selection for the type of the recurrent parent, if based on moderate-sized populations, is equivalent to one or two additional backcrosses in a continuous series. After the third backcross, however, the population resembles the recurrent parent so closely that selection is largely ineffective except for the character being transferred. The apparent recovery of the recurrent parent at this point has proved a delusion to some plant breeders. Failures of programs based on so few backcrosses are exactly what should be expected on theoretical grounds. At the California Station as many as 10 backcrosses have been made when population sizes were small and little or no selection was practiced.

While backcrossing in a continuous series requires fewer plants than any alternative mating system to transfer a monogenic dominant allele, the system has not been used at the California Station because other considerations outweigh this advantage. The system usually followed calls for growing F_2 and F_3 populations after the first, third,

Table 1.—The development of Baart 38 wheat.

Year	Nursery	Generation	Remarks	No. of nursery rows*	No. of plants used for crossing†	No. of hybrid seeds made
1930	Spring	Hope×Baart	Original cross	1	1	20
1931	Spring	Hope×Baart ^{2}	First backcross	1	4	49
1931	Summer	Hope×Baart ^{3}	Rust test; 3 heterozygous resistant plants used for second backcross	4	3	66
1932	Spring	Hope×Baart ^{4}	All 60 plants were used for third backcross	3	60	904
1932	Summer	F_1	Rust test; 31 heterozygous resistant plants selected	60		
1933	Spring	F_2	Without benefit of rust test, selected 405 plants on basis of agronomic characters	31		
1933	Summer	F_3	Rust test; selected 180 plants from homozygous resistant rows	405		
1934	Summer	Hope×Baart ^{5}	Subjected F_4 to rust, selected 14 plants for fourth backcross	180	14	240
1935	Spring	(Hope×Baart ^{5}) ×Baart 35‡	Selected 28 plants for fifth backcross to Baart 35	14	28	588
1935	Summer	F_1	Selected 290 plants	28		
1936	Spring	F_2	Inoculated with bunt, saved 1056 bunt-free plants	290		
1936	Summer	F_3	Rust test; grew 1056 lines in 3-row blocks, 340 were homozygous for resistance	3,168		
1937	Spring	F_3	Remnant seed of 340 stem-rust-resistant lines inoculated with bunt 157 were homozygous for bunt resistance. These were bulked as Baart 38.	1,020		

* Rows of Baart grown for checks and parents excluded. Rows were 16 feet long.
† Excludes Baart parent.
‡ Baart 35 is a bunt-resistant strain of Baart developed independently from Martin × Baart[7].

and sixth backcrosses. Theoretically, a minimum of 53 plants from backcrossed seeds, 96 F_2 plants, and 68 F_3 rows are required to complete the transfer through 6 backcrosses. These numbers are based on a probability of 0.999 of having at least one *Aa* plant after each backcross and at least one homozygous *AA* progeny in F_3. Somewhat smaller total populations are required with incompletely dominant or recessive genes because homozygotes can be recognized in the F_2 generation, making F_3 progenies unnecessary. In species where artificial hybridization is difficult, the number of hybrid seeds required may be reduced by growing F_2 and F_3 populations after each backcross and crossing on homozygotes.

These calculations assume that the recurrent parent consists of a single pure line, an assumption which would rarely be justified. Since varieties of self-pollinated crops ordinarily consist of a mixture of closely related pure lines, insurance that a backcross-derived variety will have the same response to environment as its prototype can be obtained only by recapturing this diversity. The danger of restricting the base to a single line can be illustrated by the performance of pure lines of a lima-bean variety. Two pure lines out of 132 involved in the purification of the variety Wilbur proved deficient in ability to emerge under the adversity of an abnormal season. Neither of these lines would have satisfactorily represented the variety of which it was a part. In order to avoid such difficulties, a number of plants of the recurrent parent are used in making the final backcross, and a large number of F_3 lines are bulked for the seed increase of the improved variety.

For the most part, the California breeding system has been based on rather large numbers of backcrossed seeds to provide for selection toward the recurrent parent in the early generations and for selection for the maximum expression of the character under transfer. Bulking of a number of lines after the final backcross is always practiced. Pugsley (7), who uses continuous backcrossing and small populations, is experimenting with the efficiency of that approach in comparison with the method described above.

Theoretical considerations coupled with the experience gained in numerous backcross programs thus establish that the three basic requirements of backcross breeding are ordinarily easily satisfied. An example of a backcross program will illustrate some of the points discussed above and point out some of the details of the California system.

Breeding of Baart 38

The procedures followed at the California Station are essentially the ones outlined by Briggs (1) with modifications to meet the requirements of the problem or to suit the convenience of the breeder. In wheat breeding, the summer nursery, planted in July and harvested in October (the normal nursery is planted in November and harvested in June), is one modification which

has been especially useful in testing for resistance to rust and also in reducing the time required for breeding stem-rust-resistant varieties. The steps taken in transferring stem-rust resistance from Hope to Baart, and merging this with bunt resistant Baart to form Baart 38, are shown in table 1. Because of the urgent need for rust resistance in this crop at the time, the first three backcrosses were made in succession. Hence the program in this respect is not typical. Rigid selection for Baart characteristics was practiced, especially during the period 1931–1934. Baart 35, resistant to bunt, was used as the recurrent parent for the fifth backcross, thus merging resistance to the two diseases.

In the summer of 1936, 3-row blocks of 1,056 F_3 families were subjected to a rust epiphytotic. In the spring of 1937, remnant seed of the 340 families homozygous for resistance to rust were tested for resistance to bunt, again in 3-row blocks. One hundred fifty-seven lines resistant to both diseases were bulked to form Baart 38. Final testing for resistance to the two diseases was thus combined with the first seed increase. The entire program, including part of the first seed increase, required the growing of slightly more than 5,000 nursery rows over a period of 8 years. A total of 1,867 hybrid seeds were made requiring the emasculation and pollination of about 125 spikes. Baart 38, it should be noted, has one less backcross than the minimum suggested earlier. Subsequently, two additional backcrosses were added and the product released as Baart 46.

ADDITIONAL ASPECTS OF BACKCROSS BREEDING

In the previous section it was established that the three requirements basic to backcross breeding are readily satisfied in the case of the transfer of monogenic characters. In this section consideration will be given to the accumulation of genes governing a single character, problems imposed by linkage, the transfer of complexly inherited characters, application of the method to cross-pollinated species, and some other features of backcross breeding.

Transfer of More Than One Gene

Although the backcross method has been used most frequently for the transfer of monogenic characters, it offers a number of procedures by which the improvement of a variety with respect to two or more characters, or the accumulation of additional desirable alleles governing a single character, can be accomplished. The use as the recurrent parent of a variety which has been improved with respect to some character will, of course, automatically preserve that improvement. Thus, Atlas 46, which is resistant to mildew and scald, is being used as the recurrent parent in breeding net-blotch-resistant Atlas. If not available at the initiation of a program, a backcross-improved strain may be substituted for its prototype part way through the program. This was done in breeding hessian fly-resistant Big Club 43. As soon as Big Club 40, resistant to bunt and stem rust, became available, it was substituted for Big Club as the recurrent parent in breeding for hessian fly resistance. Another approach is to conduct two concurrent backcross programs and merge the end products, as in the case of Baart 38. Finally, two or more genes may be transferred simultaneously in the same program. Although this procedure has occasionally been followed at the California Station, it has certain disadvantages. In the first place, somewhat larger populations are required to transfer two genes together than to transfer them independently This disadvantage is even greater with three or more genes because genetic complexity increases expotentially with the number of genes. Secondly, experience has shown that conditions allowing expression of one of the characters may not always occur, thus delaying both transfers. There is an added advantage with separate transfers, when, because of favorable circumstances, one transfer can be completed in time to serve as the recurrent parent for the other.

When two desirable linked genes are to be transferred from one variety to another, genetic considerations indicate that the linkage would aid the program. Consideration must be given, however, to the fact that it is desirable to eliminate the segment of chromosome between the two loci, especially if the donor parent is a poor one. Except for the unlikely occurrence of strategically placed double cross-overs, the elimination of such a segment can be accomplished only if cross-overs are selected early in the program, the genes carried separately, and recombined at the end of the backcrossing. Unless this is done, an otherwise unnecessary evaluation of the derived type may be required.

Frequently the need arises to accumulate in one variety several genes affecting a single character. Such transfers are in general more difficult to handle than transfers of separate characters, but they can be accomplished satisfactorily by one or more of the procedures discussed above. Although Hope wheat is not resistant to some of the presently important races of stem rust in Australia, Pugsley (7) used White Federation 38, which carries Hope resistance, as the recurrent parent in transferring the resistance to one of the Kenya strains to White Federation. He has also made use of a different type of Kenya resistance in developing White Federation 50. In this manner, insurance was obtained against the advent of new races which might attack Kenya resistance, but not Hope resistance.[3]

Many plant breeders (see 5, 6, 13) advocate the solution of the problem of new races of pathogenic organisms by the development of a series of varieties with different sources of resistance. They recommend either that a new resistant variety be substituted as soon as the previous one in the series breaks down, or that several be grown in the area to spread the risk associated with predominance of a single type of resistance. The backcross breeder would accumulate all of the genes required in the few varieties necessary to service the area. This is the only reasonably permanent solution to the problem.

Breaking Linkages by Backcrossing

The effect of the linkage of an undesirable with a desirable allele is to reduce the number of acceptable individuals in segregating populations. This restriction upon selection usually delays ordinary breeding programs until the desired linkage phase can be recovered by selection in progenies derived from appropriate genotypes. In contrast, backcross programs will not be delayed unless the linkage remains unbroken through the last backcross. In that event, release of the new type need wait only until the favorable linkage phase is obtained either by further backcrossing or by growing appropriate selfed progenies after the last backcross.

The problems imposed by linkage are more serious when an unexpressed, undesirable allele is linked with a desirable allele for which selection is practiced. The association between susceptibility to Victoria blight and resistance to crown rust in oat hybrids involving the variety Victoria is either of this type, or possibly pleiotropic in nature. Selection for resistance to both diseases could not be

[3] Private communication.

practiced because Victoria blight was recognized as a disease of oats only after the many varieties derived from Victoria had become widespread. When situations of this sort result from linked genes, the proportion of individuals in which the unfavorable linkage phase persists will, in hybridization and selection programs, be given by 1—p, where p is the recombination value. With backcross breeding on the other hand, 97% of the derived lines should not carry the undesirable allele after six backcrosses, assuming independent inheritance. This proportion should decrease to 67, 41, 10, 5, and 0.5% as the genetical map distance progressively decreases through 0.20, 0.10, 0.02, 0.01, and 0.001 units respectively. If a number of backcross-derived lines were bulked to form the new variety, the length of chromosome derived from the donor parent should vary, describing a normal distribution about the mean of 1/n. Hence, in any well-executed backcross program, undesirable linkages may be expected to be broken in a portion of the derived lines, even if the crossover value is as small as 1% and the undesirable allele is not expressed. When, therefore, conditions allowing the expression of the undesirable allele appear, it should be possible to select from commercial fields some lines carrying both desirable alleles. If such lines are not obtained in this manner, they can be obtained by making an additional backcross. Unless they are extremely tight, undesirable linkages can be expected to cause, at the worst, only temporary difficulties with backcross-produced varieties.

Quantitative Characters

Improvement with reference to quantitative characters is a difficult problem regardless of the method of breeding used, success in all cases depending on selection. Contrasted with other methods of breeding where only a single round of selection is necessary, backcross breeding requires repeated rounds of selection. Several projects at the California Station have made it possible to assess the importance of this requirement in dealing with metrical characters by backcrossing.

The transfer by C. A. Suneson of earliness and short straw from Ramona to Baart wheat is a representative example. Starting with a difference of 10 days in maturity and 14 inches in height, he had lost only one unit of each at the end of 10 backcrosses. During the progress of these backcross transfers, it became apparent that most of the difference between the parents was governed in each case by one or two major genes, and only a small portion by minor or modyifying genes. As a result of the simple genetic situation and the advantage of relatively easy measurement of the characters, both of these transfers were little more difficult than the transfer of a "qualitative" character.

The breeding of a medium-grained rice variety is illustrative of the transfer of a somewhat more complexly inherited character. The rice variety Caloro, a short-grain type, dominates the rice acreage of California. Until the breeding of Calrose, no satisfactory medium-grain variety existed, the best available being Calady, selected from the hybrid Caloro × Lady Wright. Calrose, derived from $\text{Caloro}^4 \times$ Calady, is equal to Caloro agronomically. Despite the fact that a number of genes of minor effect govern grain length in rice, it proved a simple matter to recover the desired grain length by selecting in F_2 populations of 500–1,000 individuals after each backcross. Calrose was developed at the Rice Experiment Station, Biggs, Calif., by J. W. Jones and L. L. Davis, both formerly of the U. S. Department of Agriculture.

In a current project to increase the seed size of a limabean variety by about 10%, the donor parent has seeds about twice as large as the seeds of the variety under improvement. Genetic data indicate that the parents differ with respect to this character in at least five gene pairs. After each backcross, F_2 and F_3 progenies have been bulked and screened to isolate large seeded lines. The backcrosses have been made on F_4 plants. This problem is much simplified because all genes for large seed size do not have to be transferred. The progress made to date indicates ultimate success.

No evidence is available concerning backcross improvement in quantitative characters where success requires the transfer of a very large number of minor genes. A possible means of dealing with such characters is to establish several independent backcross lines, each slightly improved for the character being transferred. After adequate backcrossing, different lines should possess one or more different genes from the donor parent. Intercrossing many such lines, followed by intense selection, could be expected to recombine some of the desired alleles into a single line.

These successes in dealing with "quantitative" characters, and promising results with others, indicate that the backcross method is not, as commonly supposed, limited as a method of breeding only to characters which are simply inherited.

Applications to Cross-pollinated Crops

In backcrossing to improve a variety of a cross-pollinated crop, the sample of the recurrent parent must be adequate to recover the gene frequencies characteristic of the variety under improvement. Caliverde alfalfa, developed by Drs. E. H. Stanford and B. R. Houston, and resistant to bacterial wilt, mildew, and leaf spot, illustrates such a breeding program (12).

During the period of more than 100 years that Chilean-type alfalfa has been grown in California, it has been moulded by natural selection until the present California Common represents a type of exceptional merit. However, it is susceptible to bacterial wilt and certain foliar diseases. Mildew- and leaf-spot-resistant plants found in California Common were used as a source of resistance to those diseases. Wilt resistance was transferred from Turkestan, a type wholly unsuited for production in California. About 200 plants of California Common were used to represent the recurrent parent in each of the 4 backcrosses. Although tetrasomic inheritance may have been involved, it necessitated only minor modifications in procedures, and it did not adversely affect the end result. Caliverde is indistinguishable from California Common alfalfa except for disease resistance.

Another application of backcross breeding to cross-pollinated crops is in connection with the improvement of inbred lines. This use of the method, however, differs in no fundamental way from its application to self-pollinated species. At present many maize inbreds are under improvement for disease or insect resistance or other characters. Since the primary purpose is the production of superior hybrids, only dominant or partially dominant alleles are useful. Convergent improvement, suggested by Richey (8), is a form of reciprocal backcrossing in which

the yielding ability of the inbreds themselves is the primary concern. It has not been particularly successful as a method of breeding, partly because of the difficulty in identifying high-yielding lines in segregating generations.

Evaluation Trials

An important feature of the backcross method emphasized by Briggs (1) is the fact that backcross-derived varieties can be released safely without benefit of evaluation trials. Suneson (14) has made yield comparisons of all the wheat varieties produced up to and including 1944. The average yield of nine backcross-derived varieties, grown in paired comparisons with their prototypes in extensive tests in nine western states, failed to show any general significant difference in yield when disease or insect injury were absent. Although none of the backcross-derived varieties developed at the California Station has been subjected to evaluation trials before its release, all varieties have filled the place predicted for them before release.

Independence of Backcross Breeding from Environment

The use of greenhouses or off-season nurseries is limited to evaluation for disease resistance and to "adding" generations in standard hybridization and selection programs, because attempts to evaluate agronomic performance are for the most part valueless under such environmental conditions. Backcross breeding does not suffer from this limitation, the only restriction being that the environment must allow the development of the character being transferred. In some cases, as many as three generations can be grown per year. Additionally, it is possible with the backcross method for the breeding to be done at a place more convenient than the area of adaptation. This may be illustrated with a program concerned with standard lima beans, a crop adapted only in the summer fog belt along the coast of southern California. Any attempt other than by backcrossing to breed a nematode-resistant standard lima at Davis, located in an area totally unsuited to the production of this crop, was doomed to failure. However, effort and expense militated against conducting a breeding program at a distance of nearly 400 miles from headquarters. A backcross program using the highly successful variety Ventura as the recurrent parent has provided an answer to this problem.

SOME ACCOMPLISHMENTS WITH THE BACKCROSS METHOD AT THE CALIFORNIA STATION

The backcross method of breeding was first used at the California Station by Briggs (1) in connection with the improvement of wheat in resistance to bunt. The reasons for adopting it were two-fold: first, the method offered a scientific approach which assured success; second, it promised to be economical of the plant breeder's time by freeing him from laborious note-taking and yield trials. This early appraisal of the backcross method proved to be correct. Although the senior author has spent only about one-fourth of his research time on plant breeding, a check of a typical period such as 1931–1940 revealed that it was possible to carry about 25 character transfers with the help of one graduate assistant.

Backcross Breeding with Wheat

During the first period of the wheat-improvement program in California, emphasis was placed on breeding for bunt resistance because this disease was an important problem in the production of the crop. This phase of the program was completed in the period 1935 to 1940, when the backcross transfer of the Martin gene to the 12 commercial varieties grown in California was completed. In the next phase of the program, another important production problem in the crop was solved, without jeopardizing the gain in bunt resistance, by transferring stem-rust resistance from Hope to the five most widely grown wheat varieties. By the further addition of hessian fly resistance to Big Club 43, this pest was eliminated from the only area where it was important.

Two of the disease-resistant varieties, Ramona 44 and Poso 44, may be regarded as "stop-gap" varieties in that only three backcrosses were used in transferring to them the stem-rust resistance of White Federation 38 and Baart 38, respectively. Two additional backcrosses were subsequently added and new strains released in 1946. Although five backcrosses were used in the breeding of Baart 38, some millers did not consider it the equal of Baart in quality. Consequently, two additional backcrosses were added to develop Baart 46. Thus, if the backcross breeder fails to achieve his goal, the remedy is simple. During the breeding of Baart 46, advantage was taken of the opportunity to select for the highest type of stem-rust resistance available in these Hope hybrids. Baart 46, therefore, has a higher level of rust resistance than the other four varieties. While not complete, the stem-rust resistance of these five varieties has been adequate to give satisfactory control of this disease.

With the solution of the most pressing production problems, the program has now entered a third phase involving further improvement with respect to bunt and stem rust resistance and also the improvement of various morphological and physiological characteristics. In the first round of backcrossing, a single source of resistance to each of the diseases in question was used. To date no races of these diseases have appeared in California which will attack the resistant varieties of wheat. In anticipation of this event, however, additional backcross programs involving disease resistance have been undertaken. The transfer to Baart of the Turkey gene for bunt resistance is almost complete. When the end-product is crossed with Baart 46, which carries the Martin gene, a strain of Baart will be obtained with resistance to all of the presently known races of bunt (2). Three additional sources of stem-rust resistance, Kenya P.I. 117526, C.I. 12633 (derived from Timopheevi wheat), and Agropyron, are being exploited. The transfer to Awned Onas of the resistance of P.I. 117526, which provides protection against all North American races of stem rust, including the 15B complex, was completed by C. A. Suneson in 1952. Dr. C. W. Schaller is adding the resistance of C.I. 12633 to Ramona, and C. A. Suneson is adding the resistance of Agropyron to Baart. In addition, White Federation 50, mentioned earlier, is available in California. Eventually double or triple resistance to stem rust will be combined in the most important varieties.

To a considerable extent, investigations dealing with morphological and physiological characters in wheat were undertaken to develop basic information. In the reciprocal

Table 2.—Backcross improvement of Baart wheat.

Designation	Pedigree	Purpose
Baart 35	Martin × Baart[7]	Bunt resistance
Baart 38	(Hope × Baart[5]) × Baart 35	Bunt and stem-rust resistance
Baart 46	Baart 38 × Baart[2]	Greater stem-rust resistance, more Baart-like
	Ramona × Baart 46[11]	Short straw
	Ramona × Baart 46[11]	Earliness
	Ramona × Baart 46[11]	Short straw and earliness
	Oro × Baart[7]	Additional bunt resistance
	Agropyron × Baart[7]	Additional stem-rust resistance
Awnless Baart	Onas × Baart[11]	Awnlessness
	Agropyron × Baart[7]	Blue kernel color
	Ramona × Baart 46*	Additional source of awnlessness
	(Onas × Baart[11]) × Baart 46[2]	Bunt and stem-rust resistant awnless Baart
Baart 52	(Red selection from Baart 38 × Baart 38[2]) × Baart 46	Red kernel color
Baart 54	Baart 46 × Baart[2]	Very Baart-like strain to be used as recurrent parent in further improvement of Baart
	Onas 53 × Baart 46*	Combine Kenya resistance to 15B stem-rust complex with the Hope resistance of Baart 46

* Program in progress with 6 to 10 backcrosses planned.

transfer of the awned vs. awnless character between the varieties Onas and Baart, the addition of awns to Onas increased the yield by about 5% and the test weight by about one lb./bu. (15). The removal of awns from Baart decreased these characteristics by an equal amount. In areas like California where awnless varieties predominate, this discovery provides a positive approach to wheat improvement, and the transfer of awns to the important awnless varieties is under way. Although initiated for academic rather than utilitarian reasons, this study has been valuable in both respects. The development of short Baart, discussed earlier, is another example of the improvement of wheat in a morphological characteristic, and the development of early Baart is an example of improvement in a physiological characteristic.

The extent and objectives for which the backcross method of breeding has been or is being used in wheat improvement at the California Station can best be visualized by reference to table 2, in which the projects involving Baart wheat, only 1 of the 12 varieties under improvement, are summarized. It should be noted that some of these projects are of more academic than practical significance. The number following the variety designation indicates the year of completion to the point of first seed increase. The superscript number indicates the number of times a parent occurs in the pedigree. Where a morphological change has resulted, this has been indicated in the name.

Backcross Breeding with Other Crops

Backcross breeding is also being used extensively in the progressive improvement of other California crops.

During the 5 years since its release, Atlas 46 barley, deriving its resistance to mildew from Hanna and to scald from Turk, has been important in stabilizing barley production in California. Schaller (10) has found that Atlas and Atlas 46 make the same yield in the absence of these diseases, but that under moderate to heavy attacks of either disease, Atlas 46 has a yield advantage of more than 15%. Soon after the release of Atlas 46, the backcross transfer of the Algerian gene was started in anticipation of the advent of races of mildew capable of attacking Hanna resistance. This program will probably not be completed in time to avoid some damage from mildew race 5, which occurred in a few barley fields in 1951 and appears to be spreading. In addition to resistance to scald and mildew, Atlas is under improvement with regard to net blotch and stripe diseases. The several other important barley varieties in California are undergoing similar improvement in their disease resistance.

In oats, both stem and crown-rust resistance are being incorporated into the dominant California varieties. The method is also being used in improving several bean varieties in resistance to root-knot nematode, to root-rots caused by *Rhizoctonia solani,* and to mosaic. Wilt-resistant flax is being developed by the same method. Much of the production of blackeyed cowpeas in California depends on backcross derived varieties resistant to root-knot nematodes and cowpea wilt. As pointed out earlier, the backcross transfers to California Common alfalfa of resistance to bacterial wilt, mildew, and Pseudopeziza leaf spot were completed in 1950.

With the stabilization of production which has resulted from the improvement of many California field-crop varieties in disease resistance, more and more attention has been directed toward unit improvements in morphological and physiological characters. The barley variety California Mariout has dull blue kernels that are at times the object of discrimination by the trade. This color is being replaced by white kernel color, which should make the variety more acceptable. In dry-edible, standard lima beans, a bright white seed coat is desirable. Ventura, the only variety which has proved agronomically acceptable in California, has a dull, grey-green seed coat. The breeding of white-seeded Ventura is partly completed. Similarly, a project to develop a large-seeded, quick-freezing variety of lima beans with a green cotyledon is nearly completed with the transfer of this character to the highly successful variety Fordhook. The breeding of smooth-awned Atlas barley was accompanied by an unexpected benefit above and beyond the advantages associated with smooth awns. Either the smooth-awned gene, or closely linked genes, cause yield to be increased by about 15% when transferred from Lion to Atlas. Projects to utilize this favorable association in improving other barley varieties in yielding ability are under way.

Success in the improvement of varieties in metrical characters, exemplified by the development of short, early Baart wheat and medium-grained rice, has stimulated further work of this type. Projects are under way to improve Atlas barley in certain aspects of malting quality, Club Mariout barley with respect to lodging, flax in oil content and iodine number, beans with respect to seed size and shape, maturity, and heat tolerance, and a number of other similar projects. Sufficient evidence is available in most instances to indicate that they can be carried to successful completion.

Additionally, a number of gene transfers of academic interest are under study in a number of crops. For example, a variety with several maturity dates is under development; similarly, a variety with a graded series of plant sizes is in process of development and another with different degrees of chlorophyll deficiency. Completion of these projects should permit more precise answers than have been possible heretofore concerning the effect of a number of morphological and physiological characteristics on agronomic merit.

SUMMARY

The backcross method of breeding is based on clear-cut genetic principles which lead with great precision to predictable results. It is surprising, therefore, that the method has not been more widely used by plant breeders.

The first consideration of the plant breeder who is contemplating the use of this method of breeding is the availability of a suitable recurrent parent. There appear to be some acceptable varieties in most of our well-established agricultural crops.

After selection of a recurrent parent, the number of backcrosses to be used exceeds all other considerations in importance. Experience at the California Station indicates that six backcrosses are usually adequate, especially when coupled with rigid selection toward the recurrent parent up to the fourth backcross. In the absence of selection, at least two additional backcrosses should be used. Growing larger than minimal populations provides the opportunity to select for high intensity of the character under transfer.

It is essential that large numbers of plants of the recurrent parent be used in the last one or two backcrosses in order to recapture the variability characteristic of that parent.

The method is easiest to apply in improving self-pollinated varieties with reference to a single character, which in turn depends on a single gene pair. If additional genes are needed for the same character (almost a certainty in breeding for disease resistance) or if genes are needed for other characters, independent backcross programs, followed by merging the end products, will best handle the problem. When a variety has been improved with respect to one or more characters, the improvements will be preserved automatically by use of the derived type as the recurrent parent in further breeding. Thus, the method provides for continuous improvement. Furthermore, it does not permit the introduction of weaknesses not characteristic of the recurrent parent. Moreover, it eliminates the necessity for expensive evaluation trials.

The method applies equally well to the improvement of cross-pollinated crops if enough crossed seeds are made to reproduce the gene frequencies characteristic of the recurrent parent.

The use of recurrent backcrossing for improving crops in characters dependent on numerous genes is limited only by the ability of the plant breeder to select for a worthwhile intensity of the character. This is the chief limitation in dealing with such characters, regardless of the method employed. In order not to dissipate the desired genes, each backcross must be followed by rigid selection. Completion of three projects of this type and promising results with others indicate the method is well suited for dealing with metrical characters.

Frequently, the most important limiting factors of production in crop varieties are obvious deficiencies governed by one or a few genes. These major objectives are placed foremost in backcross breeding, permitting the breeder to deal with simple genetic situations since all other genes are cared for automatically by the recurrent backcrossing. In this manner, it is possible to circumvent the complexities of inheritance encountered in nearly all hybrids, and the resultant difficulties which frequently overwhelm the breeder who deals with uncontrolled segregation.

LITERATURE CITED

1. Briggs, F. N. Breeding wheats resistant to bunt by the backcross method. Jour. Amer. Soc. Agron. 22:239–244. 1930.
2. ————, and Holton, C. S. Reaction of wheat varieties with known genes for resistance to races of bunt, *Tilletia caries* and *T. foetida*. Agron. Jour. 42:483–486. 1950.
3. Harlan, H. V., and Pope, M. N. The use and value of backcrosses in small grain breeding. Jour. Hered. 13:319–322. 1922.
4. Harland, S. C., and Atteck, Olga M. The genetics of cotton. XIX Normal alleles of the crinkled mutant of *Gossypium barbadense* L. differing in dominance potency and an experimental verification of Fisher's theory of dominance. Jour. Gen. 42:21–47. 1941.
5. Martin, J. H. Tailor made sorghums for industry and agriculture. Crops and Soils 3:20–23. 1951.
6. Murphy, H. C. There'll be more new oats. Iowa Farm Science 3:9–10. 1948.
7. Pugsley, A. T. Backcrossing for resistance to stem rust of wheat in South Australia. Emp. Jour. Exp. Agric. 17: 193–198. 1949.
8. Richey, F. D. The convergent improvement of selfed lines of corn. Amer. Nat. 61:430–449. 1927.
9. Riddle, O. C., and Baker, G. A. Biases encountered in large scale yield tests. Hilgardia 16:1–14. 1944.
10. Schaller, C. W. The effect of mildew and scald infection on yield and quality of barley. Agron. Jour. 43:183–188. 1951.
11. Smith, H. H. Differential photoperiod response from an interspecific gene transfer. Jour. Hered. 41:199–203. 1950.
12. Stanford, E. H. Transfer of disease resistance to standard varieties. Proc. 6th Int. Grassland Cong. (In Press).
13. Stevens, N. E., and Scott, W. O. How long will present spring oat varieties last in the central corn belt? Agron. Jour. 42:307–309. 1950.
14. Suneson, C. A. An evaluation of nine backcross derived wheats. Hilgardia 17:501–510. 1947.
15. Suneson, C. A., Bayles, B. B., and Fifield, C. C. Effect of awns on yield and market qualities of wheat. U.S.D.A. Circular 783. 1948.
16. Wiebe, G. A., and Briggs, F. N. The degree of bunt resistance necessary in a commercial wheat. Phytopath. 27:313–314. 1937.